PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P640PC00	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/DK 03/00401	International filing date *(day/month/year)* 17.06.2003	Priority date *(day/month/year)* 17.06.2002

International Patent Classification (IPC) or both national classification and IPC

C12N5/06

Applicant

KOBENHAVENS AMTS SYGEHUS, HERLEV et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19.12.2003	27.09.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Teyssier, B Telephone No. +31 70 340-2062

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK 03/00401

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-61 as originally filed

Claims, Numbers

1-40 received on 08.09.2004 with letter of 06.09.2004

Drawings, Sheets

1-3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2, 3, 9-23, 30-38
	No:	Claims	1, 4-8, 24-29, 39, 40
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-40
Industrial applicability (IA)	Yes:	Claims	1-40
	No:	Claims	-

2. Citations and explanations

see separate sheet

Reference is made to the following documents:

D1 WO 98/30676 A (Demtek AS) 16 July 1998, cited in the application

D2 US 4,892,830 A (Baylor College of Medicine) 9 January 1990, cited in the application

Re Item I

Basis of the report

The amended claims filed with the letter of 6 September 2004 are allowable under Article 34(2)(b) PCT as they find a basis in the claims and description as originally filed.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1 discloses flexible bags for the culture of cells or tissues, especially oocytes and embryos, under controlled conditions (see pages 10-12); the bags can be filled with a variety of gas mixtures and placed in thermostated baths at different temperatures, as required by the culture protocol (last two paragraphs of page 10). This system is intended to substitute for plastic air-tight boxes or for the heavier and more expensive K-system boxes discussed at pages 4-5. The boxes of the prior art are preferably used within conventional incubators for cell or embryo culture (paragraph bridging pages 4 and 5), which provides a controlled environment. It is observed that the K-system is regarded as expensive because, in an optimal setting, at least one box is required for each maturation and fertilisation experiment as well as at least one box for each continuing experiment (page 4, lines 24-26); this implies that, in a typical experimental protocol, gametes and/or eggs are transferred from the maturation or fertilisation box to one or several experiment boxes.

An example of a conventional incubator may be found in D2, cited in the application. Such an incubator typically provides means for controlling enviroment, including the composition and pressure of the atmosphere within the air-tight chamber, ports, means for manipulating cultures within the chamber as well as workbench space for any useful laboratory instrument such as a microscope. As said *conventional* incubator are, by definition, well-known to the skilled person, it is not necessary that D1 discusses their features at length.

The bags of D1 contained within a conventional incubator represent an instance of separate, independently controllable, air-tight residence chambers contained within a main chamber; K-system boxes contained within a conventional incubator, as discussed in D1, represent another instance of separate, independently controllable, air-tight residence chambers contained within a main chamber. It is observed that a "communication port" is nothing more than an opening, which all bags, boxes and incubators mentioned in D1 provide. Thus the subject-matter of claims 1, 4-8, 24-

29, 39 and 40 is not new over D1 and over the art (K-system) discussed therein (Article 33(2) PCT). The additional subject-matter of claims 2, 3, 9-23 and 30-38 does not involve an inventive step over D1 since all the additional features of these dependent claims are known means and features, which a person skilled in cell culture would fit to the incubator and/chambers as required by his own experimental protocol (Article 33(3) PCT).

This Authority observes that the features of claims 1-38 are mostly, if not entirely, expressed in functional terms and that no details are given as to the actual, physical, features of the system. As exemplified by D1 and D2 (figures 1-8 in both), it is customary in the field of cell culture devices to illustrate an invention by way of detailed drawings and to refer extensively to those drawings in the description, in order to allow a skilled person, in this instance a mechanics engineer, to build the device as decribed. Figure 3 of the application, which is the only drawing pertaining to the system of claims 1-38, appears rather crude in this respect and is neither annotated nor discussed in the description.

Claims

1. A system for in vitro producing a mammalian pre-embryo, said system comprising
   - means for obtaining a mammalian oocyte, and
   - means for obtaining a mammalian spermatozoa, and
   - an apparatus having at least two separate air-tight chambers, for which the oxygen tension of one chamber may be changed independent of the oxygen tension of the other chamber, said at least two separate air-tight chambers constitute a main chamber and at least one residence chamber, where said at least one residence chamber are smaller than said main chamber, and are located inside the main chamber and/or are attached to the main chamber,
     - said apparatus comprising at least one entrance port capable of communicating with the means for obtaining the mammalian oocyte and/or the mammalian spermatozoa, and
     - an exit port for withdrawal of the pre-embryo, as well as
     - a communication port between said at least two chambers allowing transfer of oocyte, spermatozoa and/or pre-embryo between the chambers.

2. The system according to claim 1, wherein the means for obtaining a mammalian oocyte is a system with a needle communicating under airtight conditions with a means for transferring from needle to said apparatus, such means for transferring comprises syringe and tube.

3. The system according to claim 1, wherein the means for obtaining a mammalian spermatozoa is a system in which the oxygen tension can be controlled.

4. The system according to claim 1, wherein the atmosphere within the chambers is kept aseptic.

5. The system according to claim 1, wherein the temperature of each chamber can be regulated independently.

6. The system according to claim 1, wherein the oxygen tension of each chamber is regulated independently by adding oxygen, nitrogen, carbon dioxide, helium or

another inert gas, or a mixture of two or more of these gasses simultaneously with removing gas from the chambers, in the way that the pressure of the air is in accordance with the atmosphere.

7. The system according to claim 6, wherein the pressure of the gasses inside the chambers is slightly higher than the pressure of the atmosphere surrounding the main chamber.

8. The system according to claim 1 to 7, wherein the humidity of each chamber can be controlled and regulated to a level between 50 and 100%.

9. The system according to claim 1, wherein said entrance port and said exit port is combined to a single opening means, such as a door.

10. The system according to claim 1, wherein said entrance port and said exit port is combined in a means for transporting cell culturing means and equipment to and from the main chamber.

11. The system according to claim 10, wherein said combination of said entrance port and said exit port is an air lock.

12. The system according to claim 11, wherein said entrance port constitute an inner door of said air lock and said exit port constitute an outer door of said air lock.

13. The system according to claim 12, wherein said air lock comprises walls between said inner door and said outer door constituting a small air-tight chamber.

14. The system according to claim 13, wherein said inner door and said outer door only can be opened one at a time in the way that only one door can be open at a time, and the opening of one door can only set going when the other door is totally shut.

15. The system according to claim 14, wherein the atmosphere of said air lock can be controlled and adjusted including contents of oxygen, nitrogen, carbon dioxide, helium or another inert gas, temperature and humidity.

16. The system according to claim 15, wherein said inner door of said air lock only can open when the conditions including temperature, humidity and contents of oxygen is equal to the conditions inside the chamber which the air lock is positioned inside.

17. The system according to claim 1, wherein a microscope can be placed and used when handling the oocytes, spermatozoa and embryos.

18. The system according to claim 1 to 17, wherein a working area is obtained within said main chamber, said working area comprises a place for culturing means containing the cultured cell structures, where the cultured cell structures is observed in the microscope, and said working area comprises room for handling means.

19. The system according to claim 1 to 17, wherein a micro-insemination apparatus is placed within the main chamber

20. The system according to claim 1 to 19, wherein the main chamber comprises opening means permitting entrance to human to handle the cell culture or the equipment inside the chambers.

21. The system according to claim 20, wherein to the opening means is attached gloves. These gloves are mounted in the way that human hands can fit into the gloves and handling the cell culture or the equipment inside the chambers.

22. The system according to claim 20, wherein to the opening means is attached sticks, bars or instruments manipulated by fibre optics, by which the cell culture or the equipment can be handled.

23. The system according to claim 1, wherein the main chamber has at least one small part of its surface replaced with a membrane, said membrane is sterile and has a structure through which a needle can be stuck through, when the needle is removed said membrane fills up the area where the needle was stuck through, and no gasses or particles can diffuse through the membrane either when a

needle is stuck through the membrane or no needle is stuck through the membrane.

24. The system according to claim 1 to 23, wherein the at least two separate chambers are arranged as a main chamber and one or more smaller air-tight residence chambers.

25. The system according to claim 1, wherein, said residence chambers are air-tight and can be controlled independent of each other and independent of the main chamber according to temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide.

26. The system according to claim 25, wherein said residence chambers constitute boxes for culture containers containing cell cultures of oocyte, spermatozoa, embryo, and stem cells including stem cell lines.

27. The system according to claim 26, each box is adapted for receiving one culture container containing the cell cultures of oocyte, spermatozoa, embryo, and stem cells including stem cell lines.

28. The system according to claim 27, wherein the number of said boxes correspond to the number of development stages of the of oocyte, spermatozoa, embryo and stem cells including stem cell lines.

29. The system according to claim 28, wherein said development stages comprises at least Immature oocyt, Mature oocyt, Spermatozoa, Fertilised oocyt, 4 cell embryo, 8 cell embryo, Morula, Blastocyst and stem cells including stem cell lines.

30. The system according to claim 1 to 29, wherein the oxygen tension and pressure of each chamber or air-tight boxes can be regulated by a computer by retrieving an image of the embryo in said chamber or said air-tight boxes.

31. The system according to claim 24, wherein said air-tight boxes is portable.

32. The system according to claim 31, wherein said air-tight boxes when removed from the apparatus can be connected to means for controlling temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide.

33. The system according to claim 32, wherein said means for controlling temperature, humidity, and contents of oxygen, nitrogen and carbon dioxide is portable.

34. The system according to claim 31 to 33, wherein the wall of said boxes contain a membrane.

35. The system according to claim 31 to 34, wherein the small boxes comprises fastening means for fastening one or more cell culture containers.

36. The system according to claim 35, wherein the wall of said cell culture containers contain a sterile membrane.

37. The system according to claim 31 to 36, wherein the small boxes can be transported for at least 6 days.

38. The system according to claim 1, wherein the size of the main chamber constitute a room between 1 cm and 2 m of each wall.

39. Use of the system according to claim 1 to 38 for culturing cell cultures.

40. Use of the system according to claim 1 to 38 for culturing gametes, embryoes, blastocysts, stem cells, stem cell lines.